Leading Edge
Minireview
Cell

Proteins Kinases: Chromatin-Associated Enzymes?

Chi-Wing Chow[1,*] and Roger J. Davis[2,*]
[1]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[2]Howard Hughes Medical Institute, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA
*Contact: cchow@aecom.yu.edu (C-W.C.), roger.davis@umassmed.edu (R.J.D.)
DOI 10.1016/j.cell.2006.11.015

Protein kinases contribute to the regulation of gene expression by interacting with transcription factors that are recruited to the regulatory regions of genes. Previous studies investigated the role of protein kinases in transcription initiation. Here, we discuss new insights gleaned from recent work showing that kinases can also interact with chromatin throughout the entire transcribed region of target genes (Pokholok et al., 2006; Proft et al., 2006).

Protein kinases represent a major class of signaling molecules that can modulate gene expression in response to specific environmental cues (Figure 1). In general, these events are thought to involve the transient binding of protein kinases with binding partners leading to the phosphorylation of kinase substrates. For example, the transient encounter between protein kinase A (PKA) and the transcription factor CREB leads to CREB phosphorylation and an increase in CREB-mediated transcription. However, in some instances, an apparently stable interaction between the protein kinase and chromatin has been detected. Examples include the binding of the ATM kinase to sites of DNA damage and the association of Aurora B kinase with mitotic chromatin. Protein kinases can also associate with actively transcribed genes. For instance, the TFIIH-associated protein kinase phosphorylates RNA polymerase Pol II on its C-terminal domain. Additionally, protein kinases (such as MSK and RSK) modify chromatin architecture by phosphorylating histones. Indeed, recent studies have established that protein kinases are frequently associated with transcribed genes (Pokholok et al., 2006; Proft et al., 2006).

The recruitment of protein kinases to promoter regulatory elements may have important functional consequences. A well-studied example is Hog1p, the terminal kinase of the mitogen-activated protein (MAP) kinase pathway in budding yeast, which is involved in osmoregulation. Hog1p physically associates with the promoters of target genes (Alepuz et al., 2001; Proft and Struhl, 2002). The recruitment of Hog1p to promoters may be mediated by interactions with substrates, including the transcription factors Hot1p, Msn2/4p, and Sko1p. D domains and DEF domains, such as those found in Hog1p substrates, mediate the recruitment of MAP kinases by interacting with the common docking site of the MAP kinases. This interaction does not interfere with the kinase catalytic site and instead promotes the phosphorylation of both the tethered substrate (such as a transcription factor) and other proteins in the local vicinity (including coactivators). This type of localized phosphorylation may be a general mechanism used by many protein kinases, including Hog1p, to influence gene transcription.

Hog1p can convert the Sko1p-Cyc8p-Tup1p repressor complex on target genes into an activator that recruits the histone acetylase SAGA, the histone deacetylase Rpd3p, and the SWI/SNF chromatin remodeling complexes (De Nadal et al., 2004; Proft and Struhl, 2002). Studies of p38 MAP kinase, the Hog1p ortholog in mammalian cells, similarly demonstrate that p38 can be recruited to target gene promoters to regulate SWI/SNF chromatin remodeling complexes (Simone et al., 2004). p38 is also implicated in the MSK1/2-mediated phosphorylation of histone H3 on serine 10 (Soloaga et al., 2003). Recent analysis of the ERK/MSK protein kinases in cells stimulated with progesterone illustrates how a kinase regulates gene expression when tethered to a transcription factor (Vicent et al., 2006). The progesterone receptor interacts directly with both the ERK MAP kinases and the MSK protein kinases. This kinase assembly is tethered on the promoter of target genes and leads to increased phosphorylation of serine 10 on histone H3, which is required for progesterone-induced mRNA expression (Vicent et al., 2006). In another example, RSK is recruited by NFAT transcription factors to the PPARγ2 gene during adipogenic differentiation (Yang et al., 2005).

Together, these studies indicate that protein kinases can physically associate with the regulatory regions of target genes (such as promoters) and that these interactions are functionally significant for physiological regulation of gene expression. More recent studies extend this concept by demonstrating that protein kinases can also associate with the entire transcribed region of target genes (Pokholok et al., 2006; Proft et al., 2006). Thus, protein kinases may have a more general role as chromatin-associated enzymes than previously anticipated.

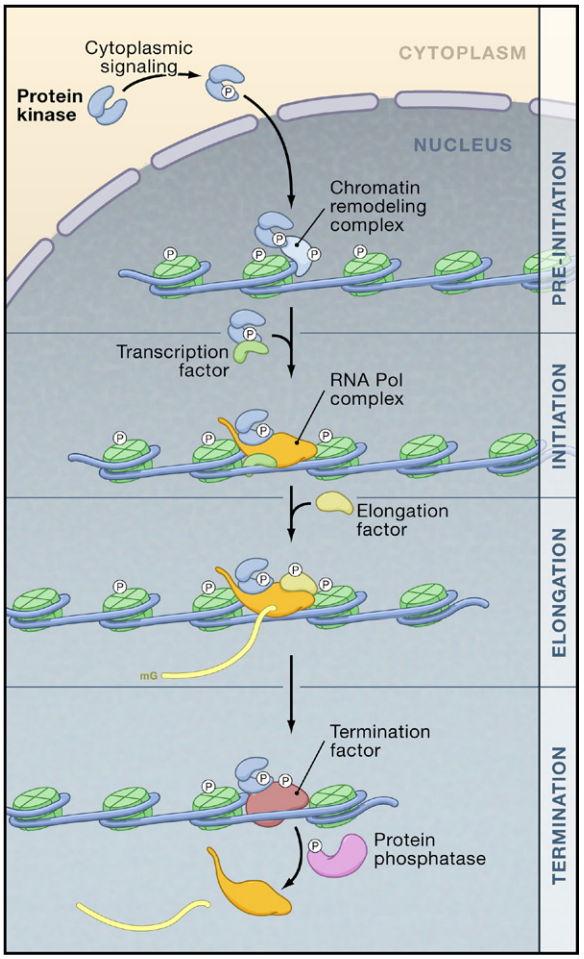

Figure 1. Regulation of Gene Transcription by Protein Kinases Tethered to Chromatin

Extracellular stimuli may activate signaling pathways that lead to the recruitment of protein kinases to target genes. The recruitment may be mediated by sequence-specific DNA-binding proteins (such as transcription factors). However, protein kinases may also be recruited to the entire transcribed region of the target genes. One possible mechanism for this is a direct association between the protein kinase and the RNA Pol II transcription elongation complex. In some instances, the 3′ noncoding region is critical for the recruitment of protein kinases to the transcribed region of the gene. The tethered protein kinases may regulate the phosphorylation of transcription factors, histones, and chromatin remodeling enzymes. Functions of tethered protein kinases may include initiation, elongation, termination, and other processes influenced by RNA Pol II.

Kinases and Transcription Elongation Factors

Alepuz et al. (2003) showed that activated Hog1p (tethered to the promoter by the Hot1p transcription factor) was capable of recruiting RNA Pol II. This recruitment promoted the expression of genes involved in the cellular response to changes in osmolarity. Recent evidence suggests that Hog1p can be photo-crosslinked to the Rpd1 subunit of RNA Pol II (Proft et al., 2006). Studies of the mammalian ortholog p38 MAP kinase also indicate an interaction with RNA Pol II (Alepuz et al., 2003). However, these are not the only protein kinases that interact with RNA Pol II. The C-terminal domain of RNA Pol II is phosphorylated on the serine 5 residues of the heptad repeats by a TFIIH-associated kinase during transcription initiation and later by CTK1 on serine 2 residues during transcription elongation (Bentley, 2005).

The interaction of Hog1p with RNA Pol II is increased when the Pol II C-terminal domain is phosphorylated (Proft et al., 2006), suggesting that Hog1p may selectively associate with RNA Pol II as a component of the transcription elongation complex. Indeed, the interaction may involve components of the transcription elongation complex, including Spt4, TFIIS, Paf1, and Thp1 (Proft et al., 2006). This finding suggests a mechanism by which Hog1p is recruited to the entire transcribed region of target genes (Sims et al., 2004).

To test whether the interaction of Hog1p with the elongation complex is functionally significant, Proft et al. (2006) uncoupled the processes of transcription initiation and elongation following osmotic stress by replacing the *STL1* promoter (which is Hot1p dependent) with an artificial promoter dependent upon the LexA-VP16 fusion protein. Osmotic stress induced the interaction of Hog1p with both the promoter and the coding region of the *STL1* gene in wild-type strains of yeast, but Hog1p was recruited only to the coding region of *STL1* in the mutant strain. Nevertheless, both the wild-type and the mutated genes exhibited Hog1p-dependent induction of mRNA expression following exposure to osmotic stress (Proft et al., 2006). These data are consistent with the hypothesis that the interaction of Hog1p with the coding region of target genes may increase mRNA expression. This function of Hog1p requires its kinase activity, although the relevant substrates are unknown.

Mechanisms of Gene Targeting by Protein Kinases

Although the recruitment of protein kinases by sequence-specific DNA-binding proteins provides a simple mechanism of gene targeting by protein kinases, it does not easily account for the recruitment of protein kinases to entire transcribed regions. For instance, Proft et al. (2006) showed that osmotic stress in yeast causes the recruitment of Hog1p to the promoter, but not the coding region, of an *STL1::LacZ* reporter gene (Proft et al., 2006). Furthermore, the recruitment of Hog1p to the coding region of *STL1* in studies using the *LexA-STL1* reporter gene showed that Hog1p recruitment to the coding region did not require the native *STL1* promoter (Proft et al., 2006). Together, these data indicate that the recruitment of Hog1p to promoters is neither sufficient nor necessary for the recruitment of Hog1p to the coding region of target genes.

What then is required for the recruitment of Hog1p to the coding region of target genes? Deletion analysis of the osmoresponsive gene *STL1* demonstrated that the recruitment of Hog1p does not depend on the major transcription factor that regulates *STL1* gene expression, the Hog1p substrate Hot1p (Proft et al., 2006). However, the recruitment of Hog1p to the transcribed region of the *STL1* gene does require the 3′ noncoding region. Remarkably, addition

of the *STL1* 3′ noncoding region to a normally nonresponsive gene conferred osmotic stress-induced recruitment of Hog1p to the transcribed region of the heterologous gene (Proft et al., 2006). However, it is not clear whether this recruitment affects the expression of the heterologous gene. Further study is also needed to elucidate how the 3′ noncoding region of a Hog1p-responsive gene can recruit Hog1p to the transcribed region of a heterologous gene.

Kinases and Specific Patterns of Gene Interactions

Can the finding that Hog1p interacts with the entire transcribed region of target genes be generalized to other proteins kinases? Pokholok et al. (2006) addressed this question by performing genomewide analyses of protein kinase interactions with chromatin. This analysis demonstrated that Hog1p exhibited osmotic stress-induced interactions with 36 genes; Hog1p occupancy was greatest at the promoter but extended throughout the entire transcribed regions of these target genes. Similar studies of the MAP kinase signaling pathway that responds to pheromones demonstrated that the terminal protein kinases in that pathway, Fus3p and Kss1p, bound to nine genes that were transcribed rapidly following exposure to pheromone. And like Hog1p, chromatin occupancy by Fus3p and Kss1p was detected throughout the entire transcribed region of these target genes (Pokholok et al., 2006). The Ste5p scaffold protein, which assembles the pheromone MAP kinase pathway, also occupied the entire transcribed regions of these target genes. This finding is surprising because it is well-established that Ste5p tethered to the plasma membrane is fully functional and that cell surface membrane recruitment of components of the MAP kinase pathway may be essential for signal initiation mediated by Ste5p. These considerations raise questions concerning the physiological relevance of the association of Ste5p with target genes. Nevertheless, these data establish that the recruitment of protein kinases to the transcribed region of target genes is not restricted to the Hog1p pathway.

In contrast to MAP kinases, which appear to occupy both regulatory and coding regions of target genes, an analysis of PKA isoforms demonstrated different patterns of gene targeting (Pokholok et al., 2006). Tpk1p occupied the entire transcribed region of actively transcribed genes in cells grown in glucose. In contrast, Tpk2p was found almost exclusively associated with the promoters of ribosomal protein genes, whereas Tpk3p was not found to associate with chromatin (Pokholok et al., 2006). The physiological significance of the association of these cAMP-dependent protein kinases with actively transcribed genes is unclear. Because Tpk3p can substitute for Tpk1p and Tpk2p in Tpk1/2p-deficient strains, one test of the physiological significance of the interaction of Tpk1 and Tpk2 with chromatin would be to compare the interaction of Tpk3 with target genes in wild-type and Tpk1/2p-deficient strains. Nevertheless, these data indicate that marked differences in gene targeting by protein kinases exist and that individual members of a closely related protein kinase family may exhibit functional differences in gene regulation.

Different Means to Target Different Genes

The protein kinase Tor1p is thought to regulate gene expression by activating downstream pathways that signal from the cytoplasm to the nucleus. However, a recent study has established that Tor1p is dynamically distributed between the cytoplasm and nucleus in yeast (Li et al., 2006). Starvation or treatment with the drug rapamycin causes the Tor1p complex 1 (TORC1) to be excluded from the nucleus. Previous studies have demonstrated that cytoplasmic TORC1 regulates RNA Pol II-dependent gene expression. Examples include Gln3p-dependent gene expression (Beck and Hall, 1999) and Ifh1p:Fhl1p-dependent ribosomal protein gene expression (Powers et al., 2004). In contrast, nuclear TORC1 appears to regulate gene expression dependent on RNA Pol I, including the expression of ribosomal RNA (Li et al., 2006). Interestingly, this nuclear function of TORC1 is mediated by the binding of TORC1 to the promoter for the 35S ribosomal pre-RNA gene. This interaction requires a putative helix-turn-helix motif in Tor1p (residues 815–837) that may contribute to DNA-binding activity (Li et al., 2006). Whether TORC1 also associates with other regions of target genes requires further study. Together, these data indicate that a single protein kinase can regulate different classes of target gene expression by both nuclear and non-nuclear mechanisms. Coordination between these mechanisms is required for correct physiological responses. A similar pattern of both nuclear and non-nuclear signaling pathways may be engaged by other protein kinases that dynamically redistribute between the cytoplasm and the nucleus.

Perspective

The discovery that signaling kinases interact with the entire transcribed region of target genes is compelling (Pokholok et al., 2006; Proft et al., 2006). However, this observation raises a number of questions that remain unresolved and must be addressed by future studies.

A weakness of the data that have been reported is that only one method, chromatin immunoprecipitation (ChIP), has been employed to establish that signaling kinases can interact with the entire transcribed region of target genes. The ChIP approach is very powerful. Nevertheless, the rigor of this method depends on the nature of the crosslinking that is performed, the specificity and efficacy of the antibody that is used for immunoprecipitation, and the ability of the investigator to fragment chromosomal DNA. Indeed, the spatial resolution of ChIP technology to distinguish between factors that interact at different sites within a gene is entirely dependent upon the quality of chromosomal DNA fragmentation. An independent analysis that uses different procedures is needed to confirm the conclusions of the ChIP studies, and methods to visualize protein kinase interactions in live cells are required to examine the dynamics of the interactions with chromatin.

An important goal for future studies will be to rigorously determine the physiological significance of protein kinase interactions with the entire transcribed region of target genes. Although the study reported by Pokholok et al. (2006) did not address this question, the question of physi-

ological significance was examined by Proft et al. (2006). These investigators demonstrated that osmotic stress in yeast caused both increased *STL1* mRNA expression and Hog1p occupancy of the *STL1* coding region in *LexA-STL1* strains. These data suggest an association between Hog1p binding to the coding region of the target gene with Hog1p-dependent expression of the target gene. However, this observation falls short as a test of the significance of the interaction of Hog1p with the transcribed region of target genes, as Hog1p occupancy of target genes may be a passive consequence of Hog1p-dependent transcription. A direct test of the hypothesis that target gene occupancy by Hog1p is functionally significant would require the demonstration that the selective loss of Hog1p occupancy causes defects in target gene expression.

The mechanism that mediates the recruitment of protein kinases to the entire transcribed region of target genes also needs to be defined. Hog1p appears to selectively interact with the RNA Pol II transcription elongation complex (Alepuz et al., 2003; Proft et al., 2006). This observation suggests that RNA Pol II may recruit Hog1p to the entire transcribed region of target genes. Whether this mechanism accounts for the recruitment of other protein kinases to target genes is unclear. In addition, the specific function of the 3′ noncoding region of the *STL1* gene in mediating Hog1p recruitment to the transcribed region of target genes has not been defined (Proft et al., 2006). Whether 3′ noncoding regions are relevant to other Hog1p-dependent target genes or to the recruitment of other protein kinases is unclear. Furthermore, it is not established whether the roles of RNA Pol II and the 3′ noncoding region reflect two aspects of the same mechanism or whether these represent independent mechanisms that recruit Hog1p to the coding region of target genes.

The extent of protein phosphorylation in vivo is a function of both the rate of phosphorylation and the rate of dephosphorylation. Therefore, an obvious prediction is that chromatin-tethered protein phosphatases may also be present. One potential example is the phosphatase Ssu72p that can regulate the ratio of serine 5 and serine 2 phosphorylation of the heptad repeats of the C-terminal domain of RNA Pol II (Krishnamurthy et al., 2004). Further studies to identify phosphatases that may antagonize the functions of chromatin-tethered kinases are warranted. In addition, it is possible that other phosphorylation-related enzymes may interact with actively transcribed genes. One example is the recent finding that glucose-regulated gene expression in plants can be mediated by the enzyme hexokinase 1 (Cho et al., 2006). These investigators demonstrated that hexokinase 1 acts as a glucose sensor, in a complex with VHA-B1/RPT5B, that integrates nutrient and hormonal signals by interacting directly with target genes. This observation indicates that proteins with known cytoplasmic functions may also be recruited to chromatin to regulate gene expression.

It is now established that protein kinases tethered to chromatin may contribute to transcription initiation and elongation. However, transcription is a complex process and it is possible that tethered protein kinases may contribute to multiple additional processes, including 5′ capping, splicing, 3′ end cleavage, polyadenylation, and transport to the cytoplasm (Howe, 2002; Svejstrup, 2004). Further studies are needed to elucidate possible roles of tethered protein kinases in these processes. It is intriguing that the chromatin structure of actively transcribed genes may enable interactions between transcription initiation, elongation, and termination (Proudfoot, 2004). We anticipate that the analysis of protein kinase occupancy of target genes will be an exciting and productive area of research over the next few years.

ACKNOWLEDGMENTS

We thank K. Gemme for expert administrative assistance. R.J.D. is an HHMI investigator.

REFERENCES

Alepuz, P.M., Jovanovic, A., Reiser, V., and Ammerer, G. (2001). Mol. Cell *7*, 767–777.

Alepuz, P.M., de Nadal, E., Zapater, M., Ammerer, G., and Posas, F. (2003). EMBO J. *22*, 2433–2442.

Beck, T., and Hall, M.N. (1999). Nature *402*, 689–692.

Bentley, D.L. (2005). Curr. Opin. Cell Biol. *17*, 251–256.

Cho, Y.-H., Yoo, S.-D., and Sheen, J. (2006). Cell *127*, 579–589.

De Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G., and Posas, F. (2004). Nature *427*, 370–374.

Howe, K.J. (2002). Biochim. Biophys. Acta *1577*, 308–324.

Krishnamurthy, S., He, X., Reyes-Reyes, M., Moore, C., and Hampsey, M. (2004). Mol. Cell *14*, 387–394.

Li, H., Tsang, C.K., Watkins, M., Bertram, P.G., and Zheng, X.F. (2006). Nature *442*, 1058–1061.

Pokholok, D.K., Zeitlinger, J., Hannett, N.M., Reynolds, D.B., and Young, R.A. (2006). Science *313*, 533–536.

Powers, T., Dilova, I., Chen, C.Y., and Wedaman, K. (2004). Curr. Top. Microbiol. Immunol. *279*, 39–51.

Proft, M., and Struhl, K. (2002). Mol. Cell *9*, 1307–1317.

Proft, M., Mas, G., de Nadal, E., Vendrell, A., Noriega, N., Struhl, K., and Posas, F. (2006). Mol. Cell *23*, 241–250.

Proudfoot, N. (2004). Curr. Opin. Cell Biol. *16*, 272–278.

Simone, C., Forcales, S.V., Hill, D.A., Imbalzano, A.N., Latella, L., and Puri, P.L. (2004). Nat. Genet. *36*, 738–743.

Sims, R.J., 3rd, Belotserkovskaya, R., and Reinberg, D. (2004). Genes Dev. *18*, 2437–2468.

Soloaga, A., Thomson, S., Wiggin, G.R., Rampersaud, N., Dyson, M.H., Hazzalin, C.A., Mahadevan, L.C., and Arthur, J.S. (2003). EMBO J. *22*, 2788–2797.

Svejstrup, J.Q. (2004). Biochim. Biophys. Acta *1677*, 64–73.

Vicent, G.P., Ballare, C., Nacht, A.S., Clausell, J., Quiles, I., Jordan, A., and Beato, M. (2006). Mol. Cell *24*, 367–381.

Yang, T.T., Xiong, Q., Graef, I.A., Crabtree, G.R., and Chow, C.W. (2005). Mol. Cell. Biol. *25*, 907–920.